Figure 1

A

CIRS X CIRS donor molecule with donor fragment
BB'   BB'

IRS Y IRS receptor molecule with receptor fragment
PP'   PP'

↓ IRS recombinase
    e.g. phage φC31 Int protein

Y donor molecule with receptor fragment
BP'   PB'

X receptor molecule with donor fragment
PB'   BP'

B

RRS-1 X RRS-2 donor molecule with donor fragment

RRS-1 Y RRS-2 receptor molecule with receptor fragment

↑↓ RRS recombinase
     e.g. phage P1 Cre protein

RRS-1 Y RRS-2 donor molecule with receptor fragment

RRS-1 X RRS-2 receptor molecule with donor fragment

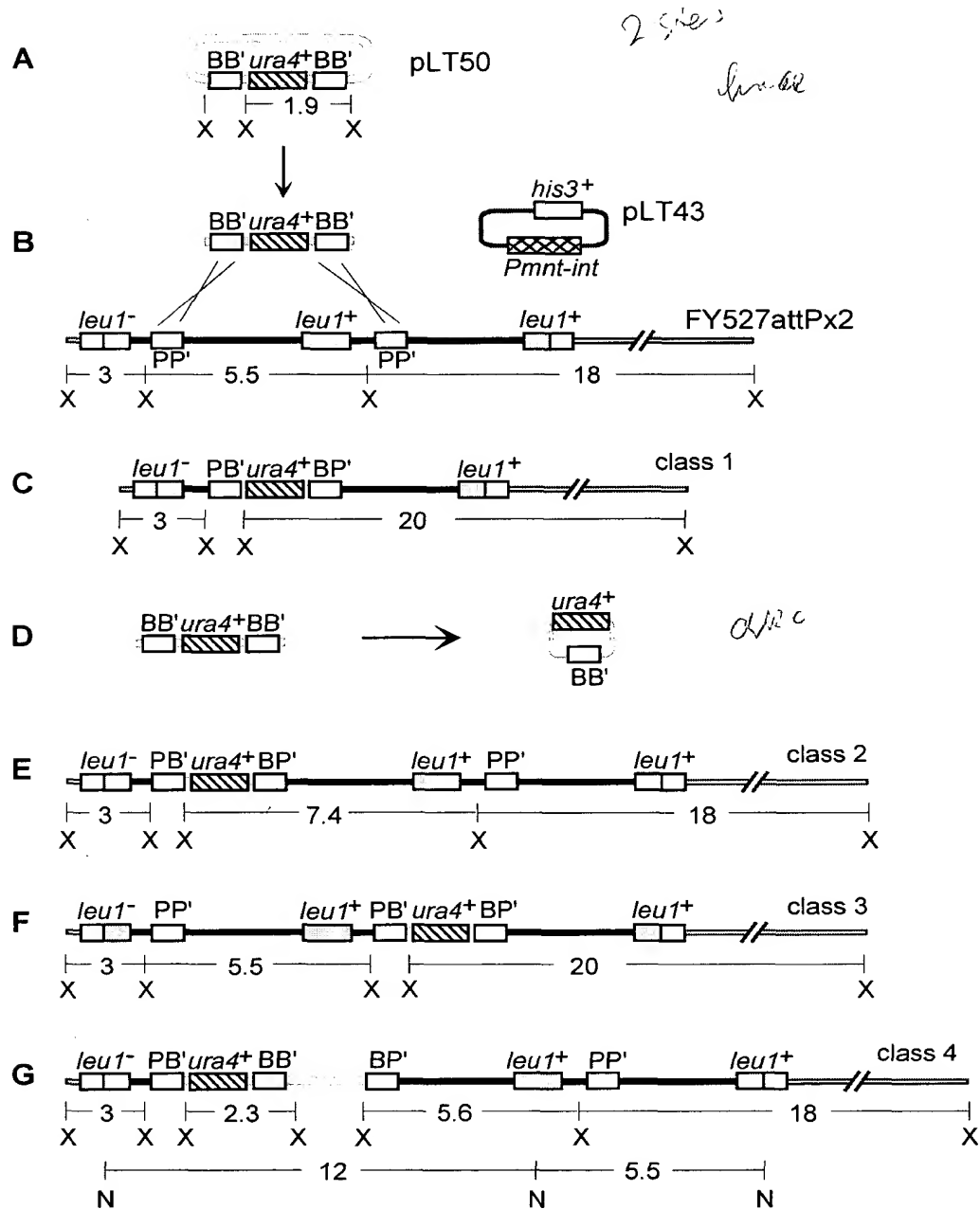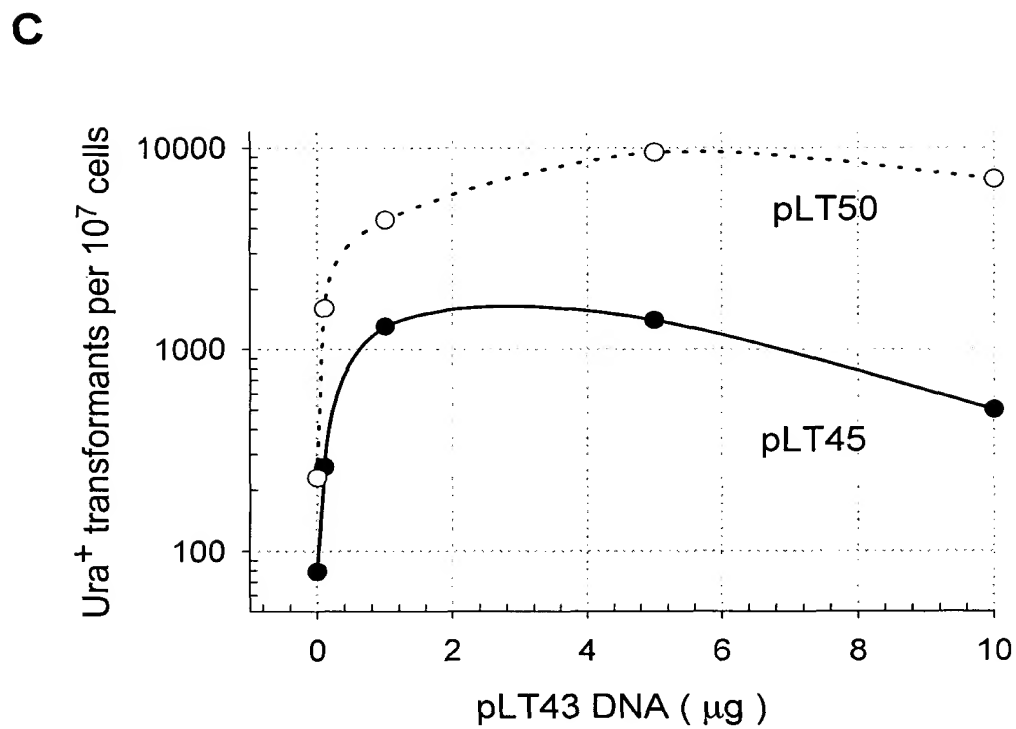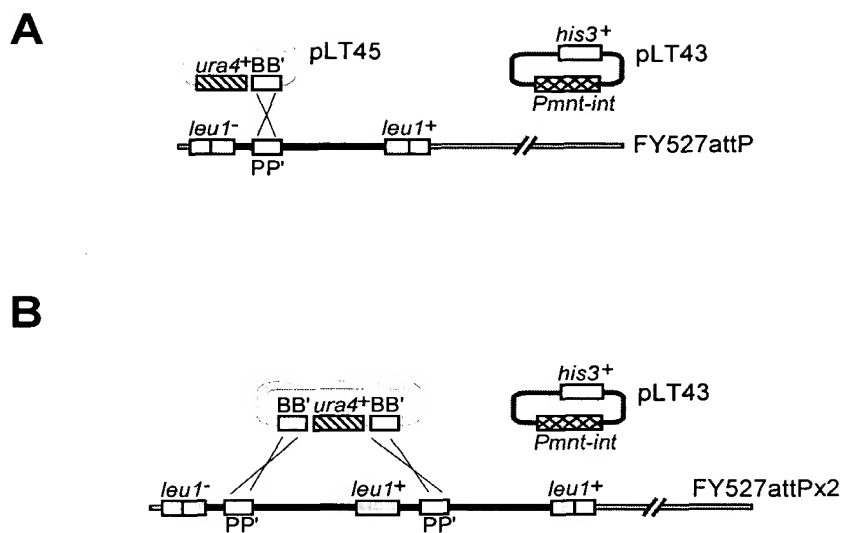|	
PP'	= *attP*
BB'	= *attB*
PB'	= *attR*
BP'	= *attL*
◀	= *loxP*
◁	= *lox511*

Figure 2

Figure 3

A

B

C

Figure 4

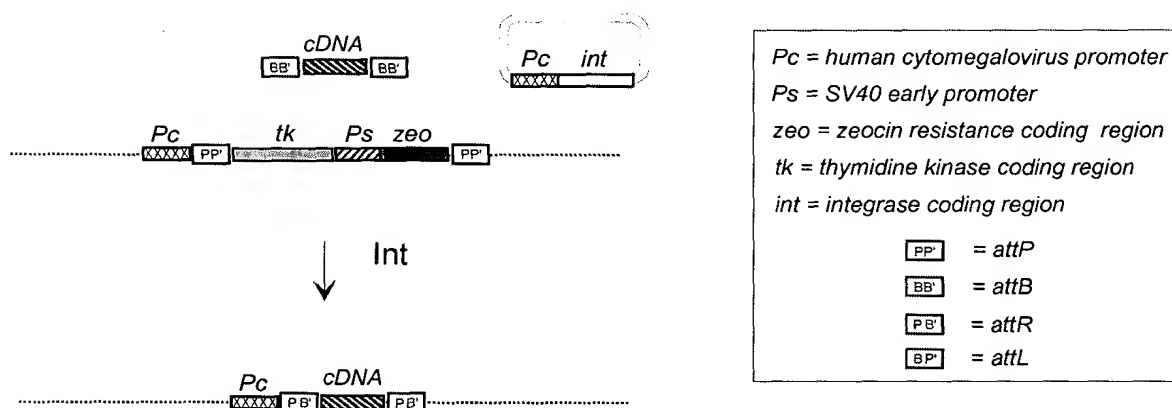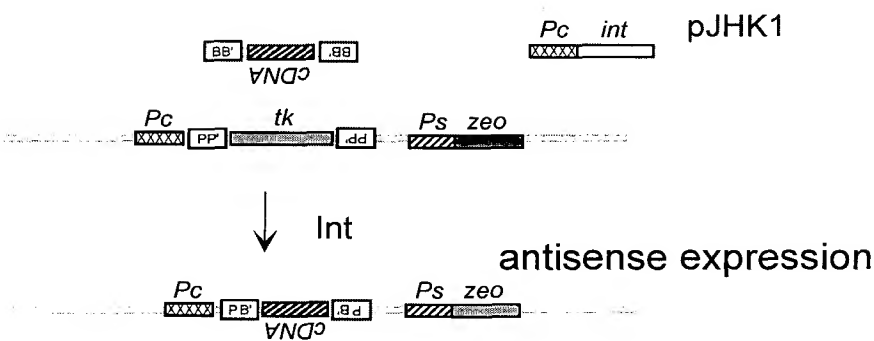# cDNA integration in mammalian cells
transient expression of *int*

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin resistance coding region
tk = thymidine kinase coding region
int = integrase coding region

PP'	= attP
BB'	= attB
PB'	= attR
BP'	= attL

Strategy for cDNA integration in mammalian cells

A

cDNA
BB' .BB   Pc int pJHK1
XXXXX

JHK3   Pc   tk   Ps zeo
XXXXX PP'   .dd   zeo

↓ Int

Pc   cDNA   Ps zeo   sense expression
XXXXX P B'   P B.   zeo

B

BB' .BB
cDNA   Pc int pJHK1
XXXXX

Pc PP'   tk   .dd   Ps zeo

↓ Int

Pc   Ps zeo   antisense expression
XXXXX P B'   P B.   zeo
cDNA

C   hpt   pJHK2
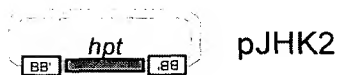BB' .BB
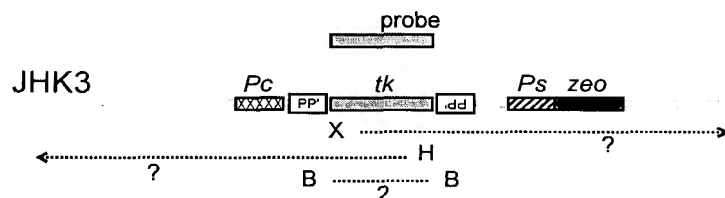
D   Pc hpt Ps zeo   pcDNA3.1-hpt
XXXXX   zeo

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin reistance coding region
tk = thymidine kinase coding region

PP' = attP
BB' = attB
P B' = attR
B P' = attL

E   Single copy target construct in human cells

probe

JHK3

Pc   *tk*   *Ps* *zeo*

X .... ⟶

⟵.. H

? .... B .... B
2

Pc = human cytomegalovirus promoter
Ps = SV40 early promoter
zeo = zeocin reistance coding region
tk = thymidine kinase coding region

[PP'] = attP
[BB'] = attB
[PB'] = attR
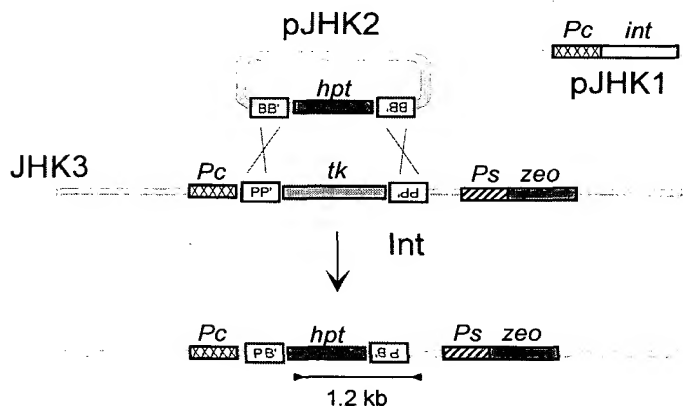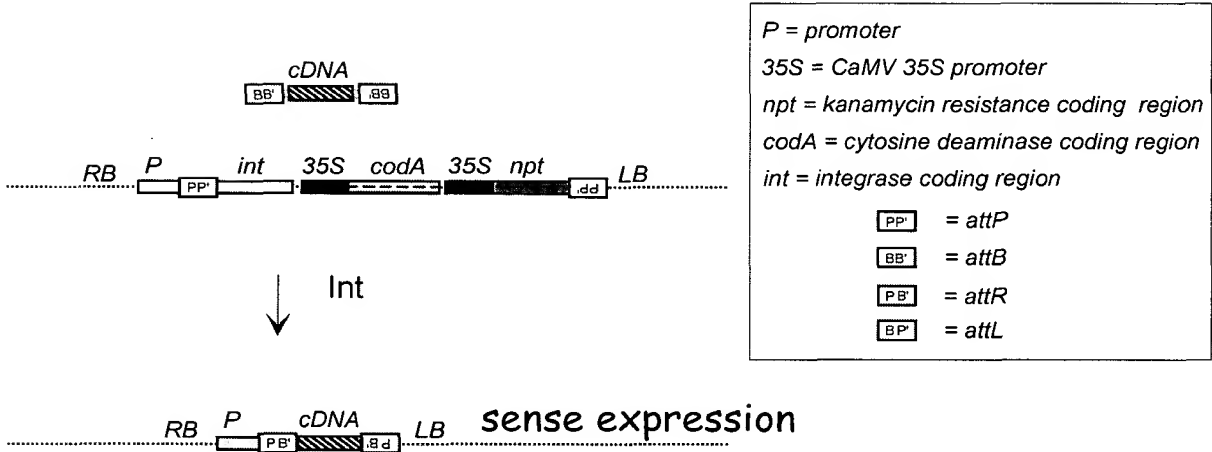[BP'] = attL

F   PCR detection of DNA exchange

pJHK2

Pc *int*
pJHK1

hpt

[BB']   [.BB]

JHK3   *Pc*   *tk*   *Ps* *zeo*

Int ⟶

Pc   *hpt*   *Ps* *zeo*

1.2 kb

Figure 6

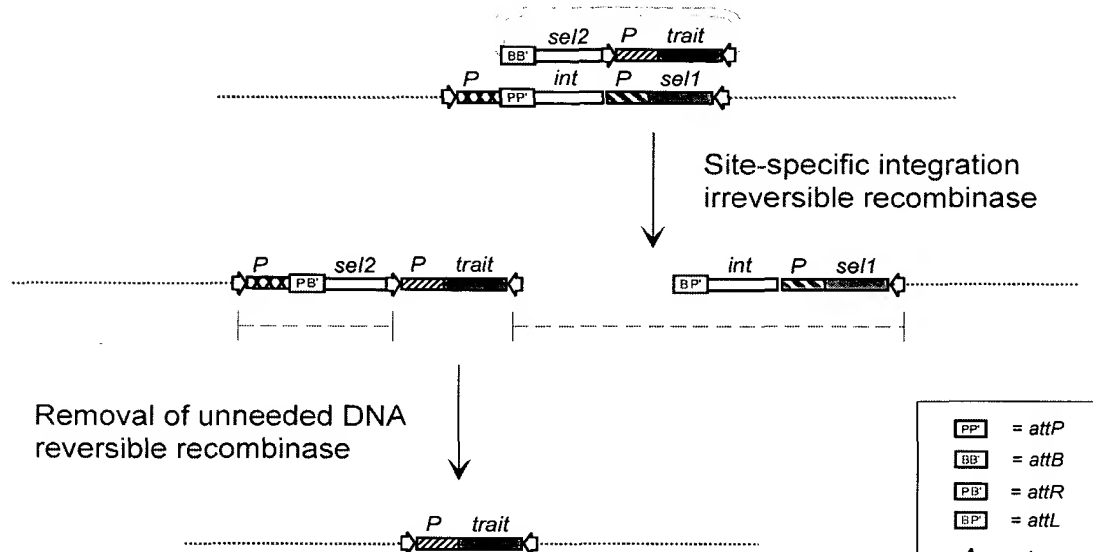cDNA integration in plant cells
int expressed from target site

A

cDNA

| BB' | //////// | .88 |

RB P   *int*   35S   codA   35S   npt   LB
| PP' |

↓ Int

RB P   cDNA   LB   **sense expression**
| PB' | //////// | .8 d |

P = promoter
35S = CaMV 35S promoter
npt = kanamycin resistance coding region
codA = cytosine deaminase coding region
int = integrase coding region

| PP' | = attP
| BB' | = attB
| PB' | = attR
| BP' | = attL

B

| BB' | //////// | .88 |
cDNA

RB P   *int*   35S   codA   35S   npt   LB
| PP' |

↓ Int

RB P   LB   **antisense expression**
| PB' | //////// | .8 d |
cDNA

Figure 7

General strategy to incorporate only the trait gene
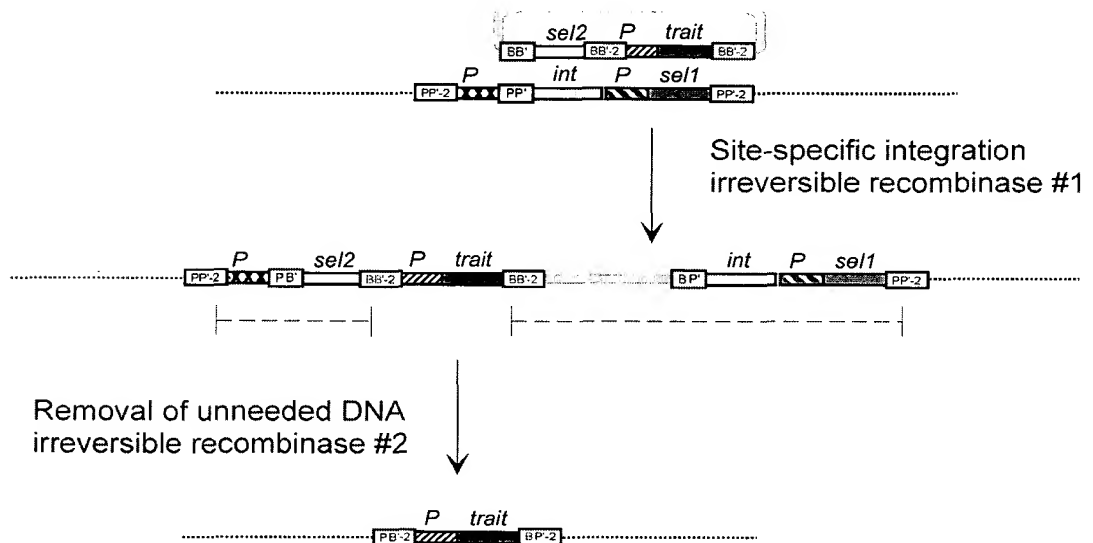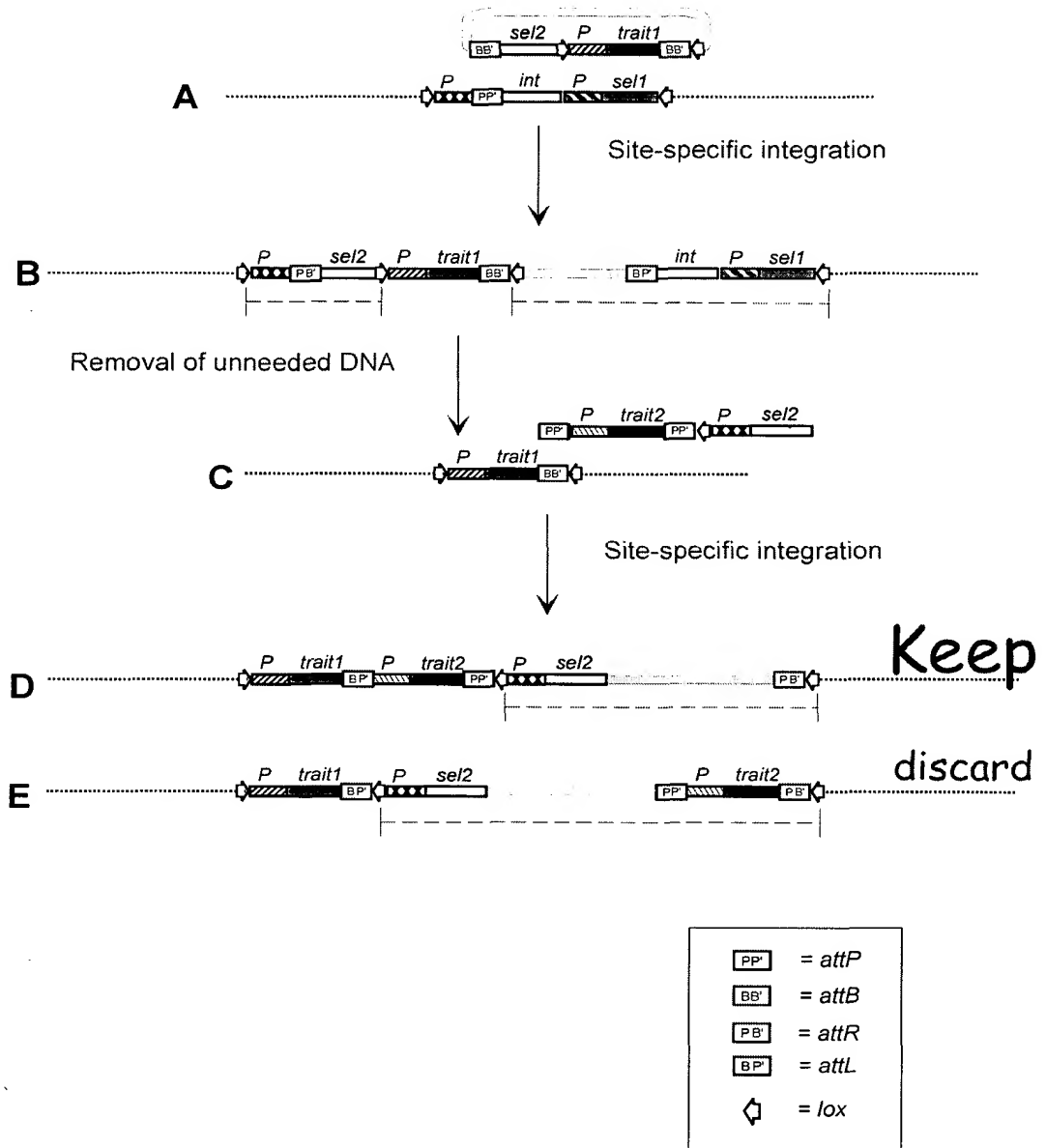
General strategy to stack genes, part1

Use of directly oriented sites

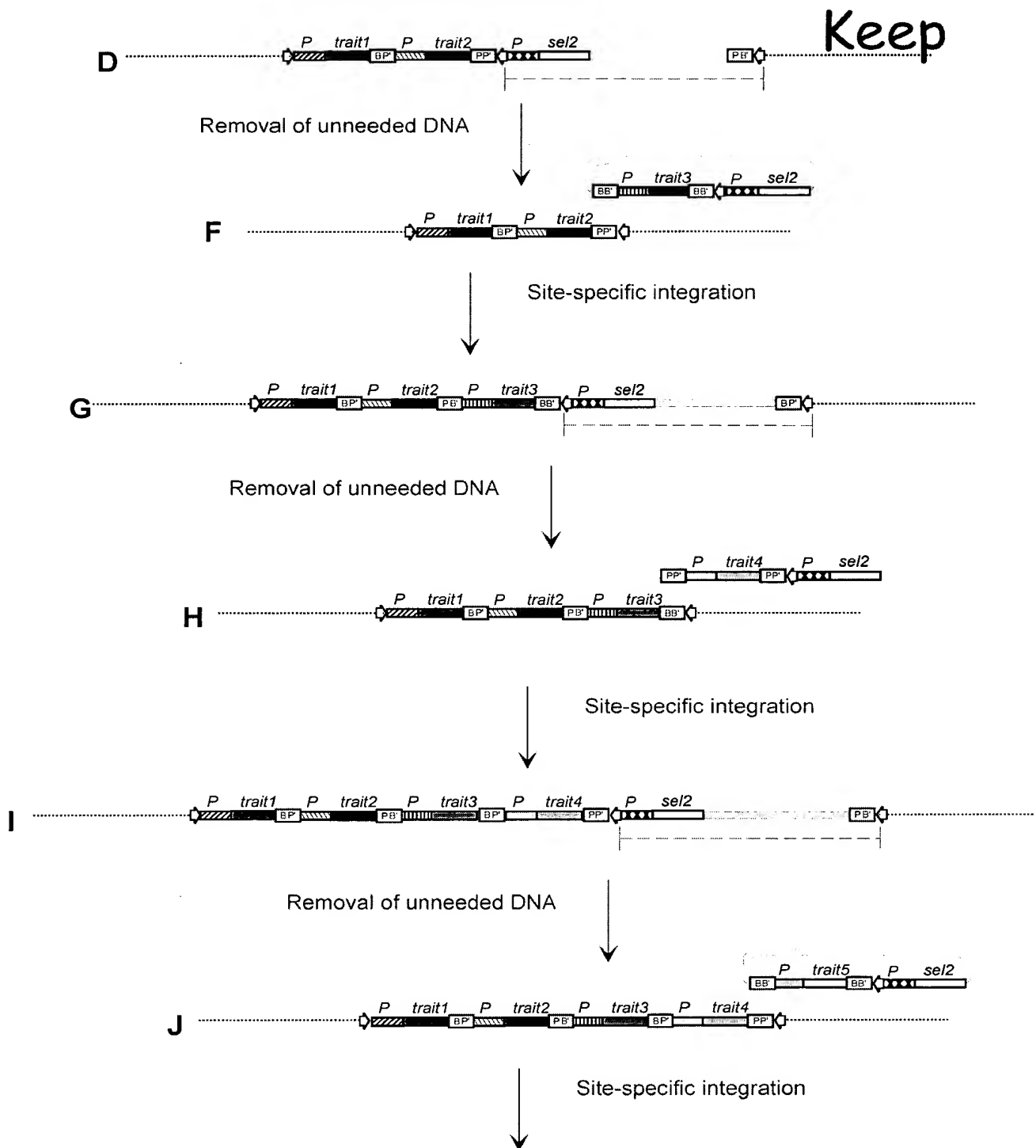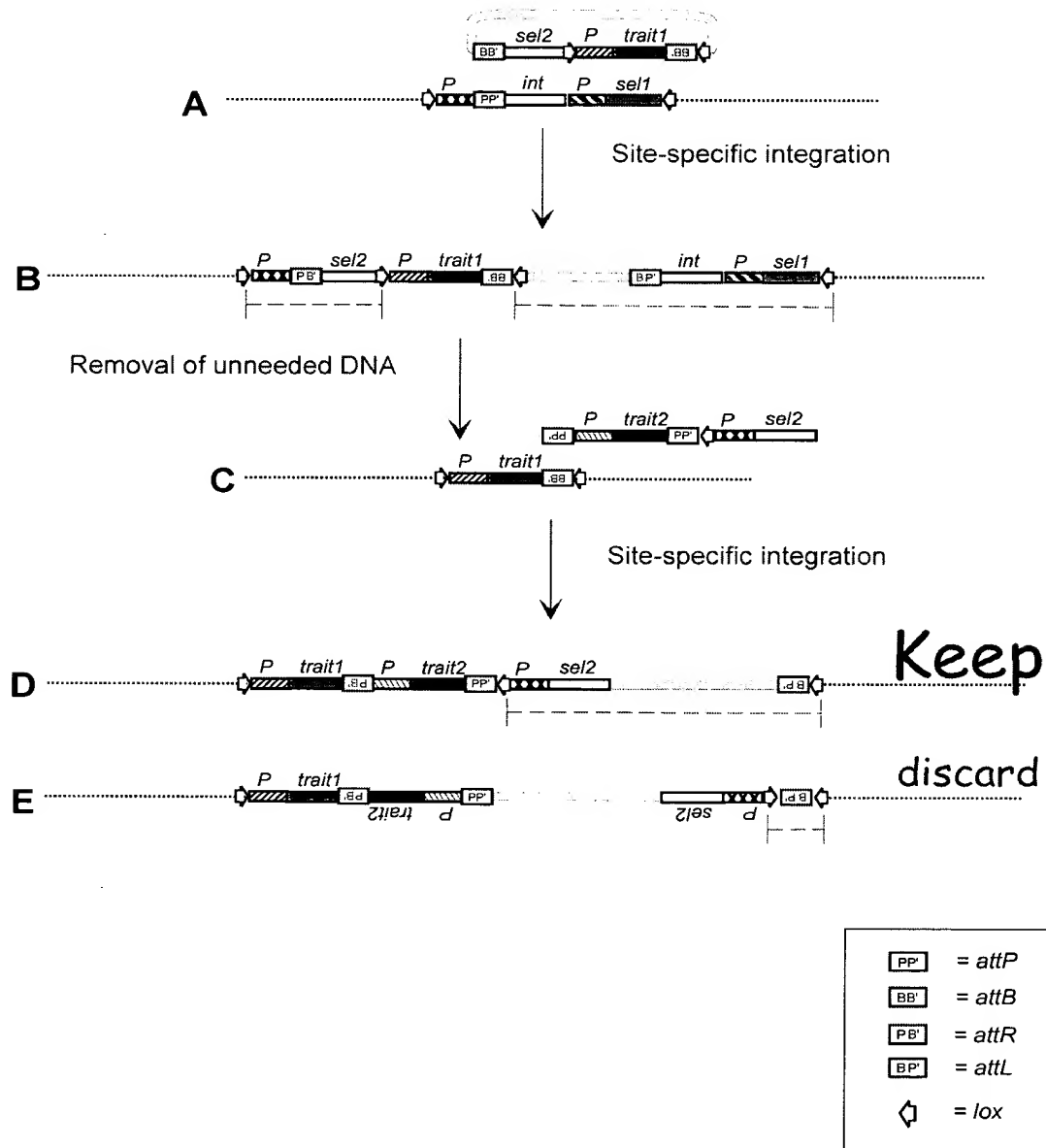General strategy to stack genes, part2
Use of directly oriented sites

Keep

D

Removal of unneeded DNA

F

Site-specific integration

G

Removal of unneeded DNA

H

Site-specific integration

I

Removal of unneeded DNA

J

Site-specific integration

General strategy to stack genes, part1

Use of inverted sites

PP'	= attP
BB'	= attB
PB'	= attR
BP'	= attL
◊	= lox

General strategy to stack genes, part2
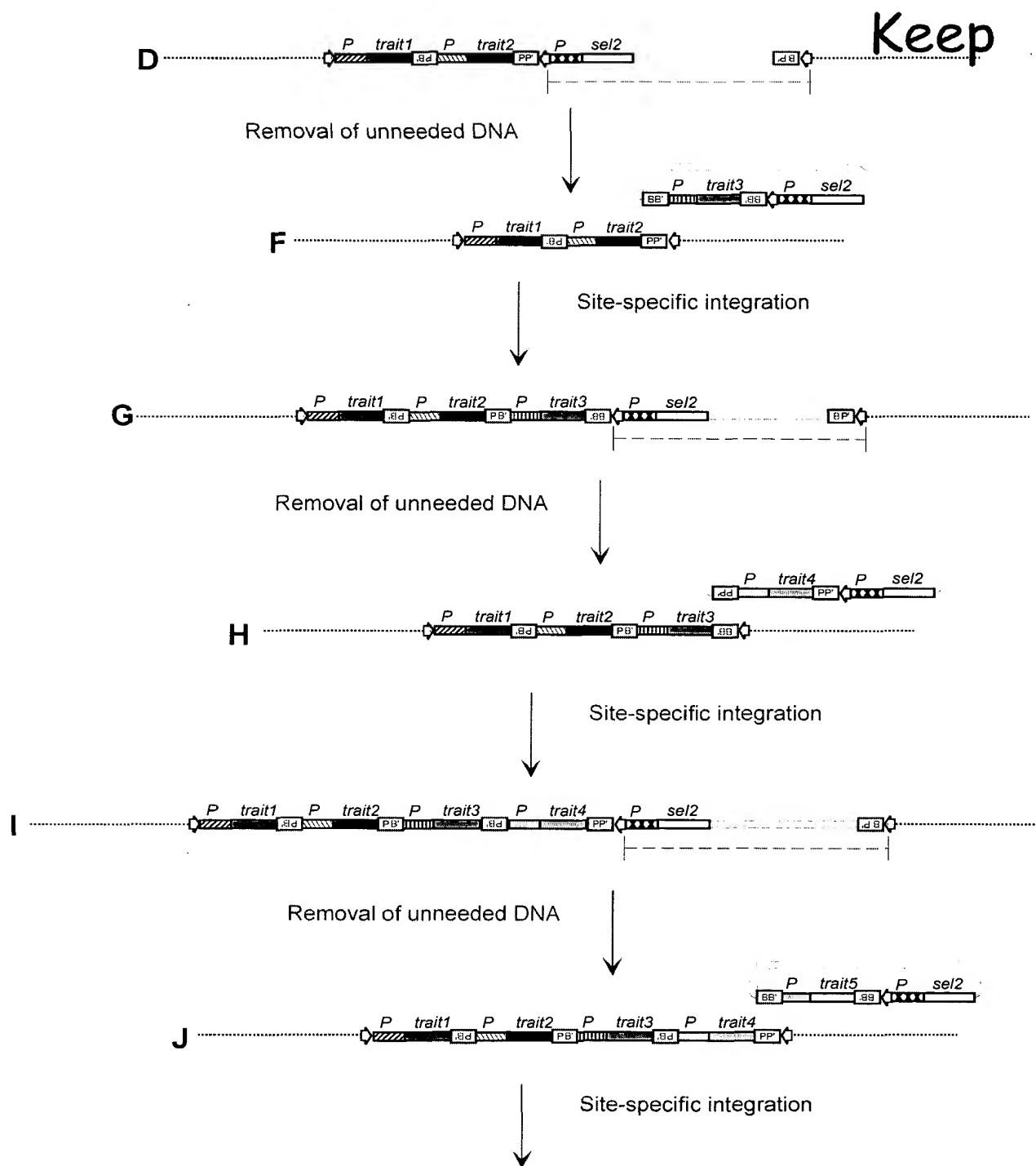
Use of inverted sites

Keep

Figure 10

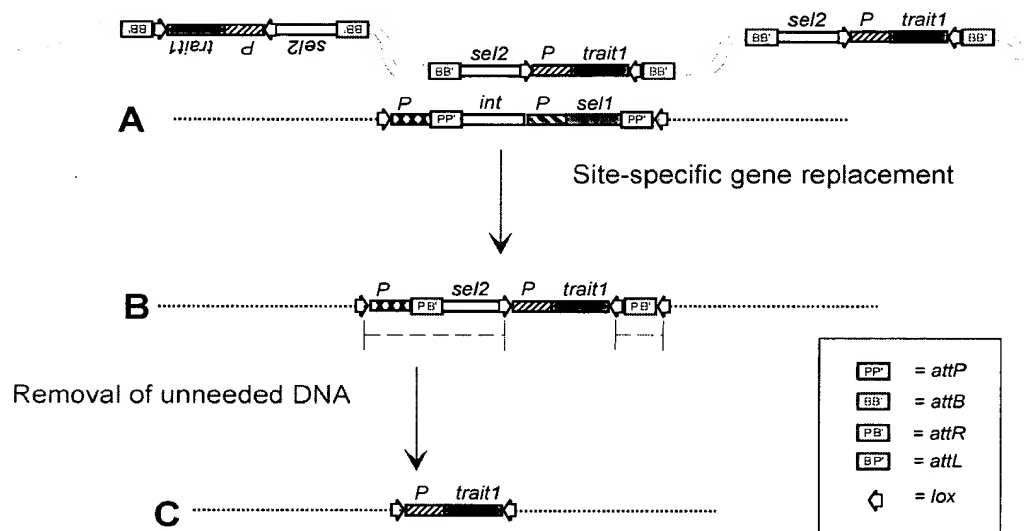Gene replacement in the host genome with directly oriented dual sites

Figure 11

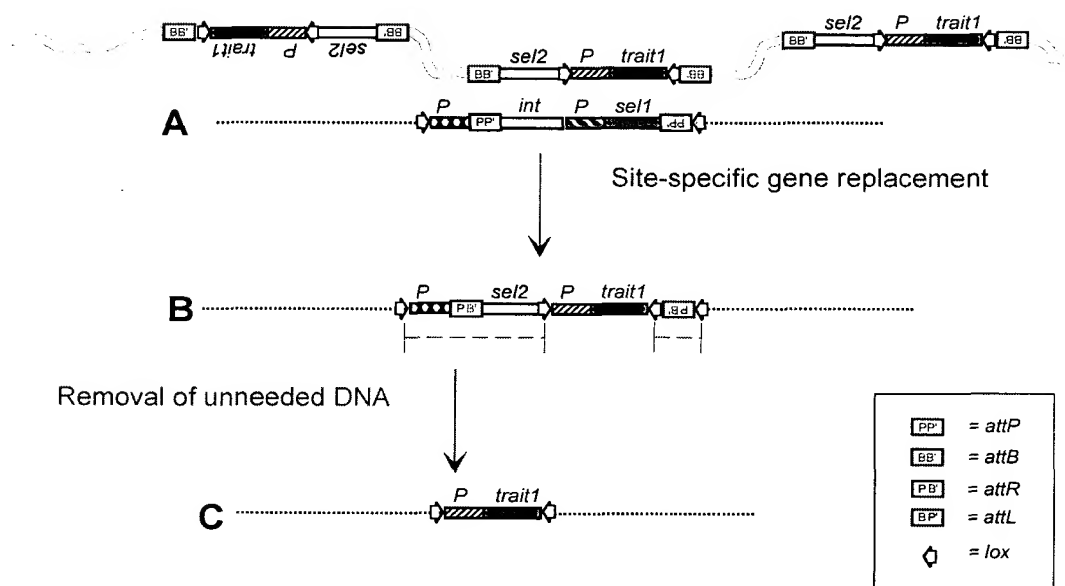# Gene replacement in the host genome with inverted dual sites

Site-specific gene replacement

Removal of unneeded DNA

PP'	= attP
BB'	= attB
PB'	= attR
BP'	= attL
◊	= lox

Figure 12

Transgene translocation from one chromosome to another

Figure 13

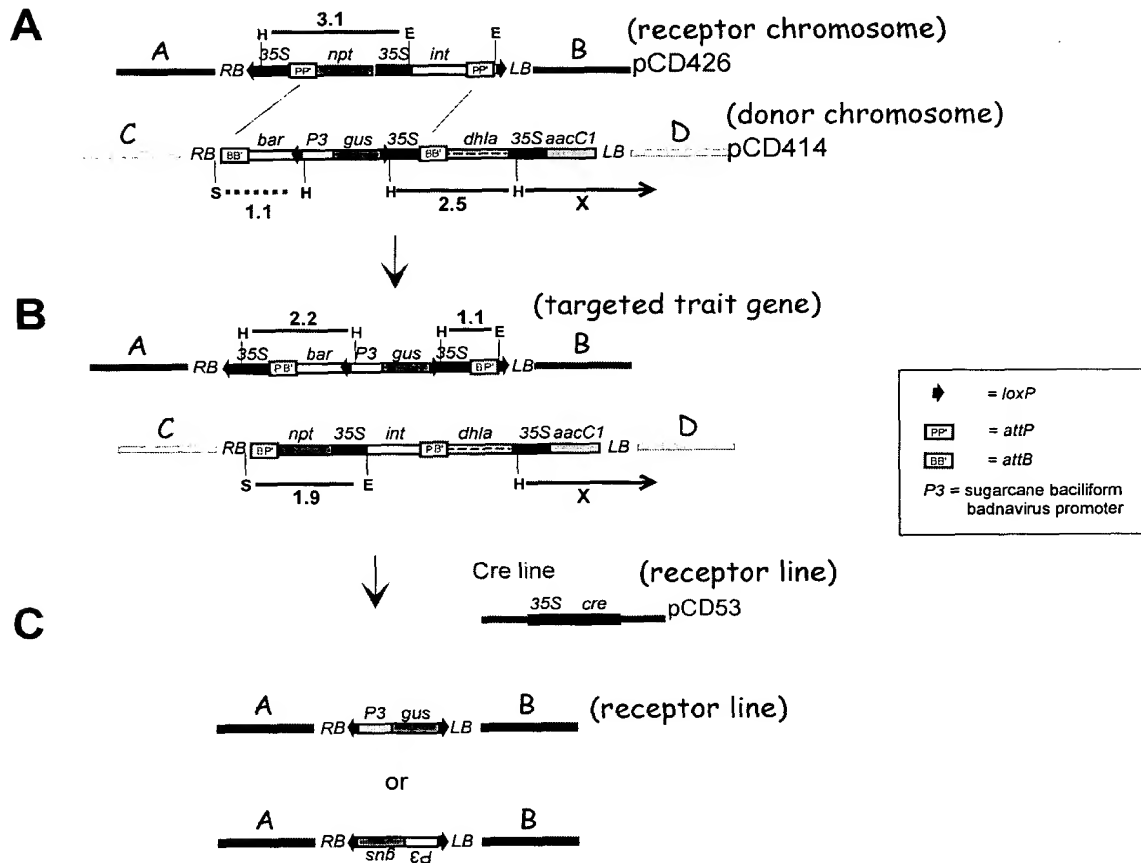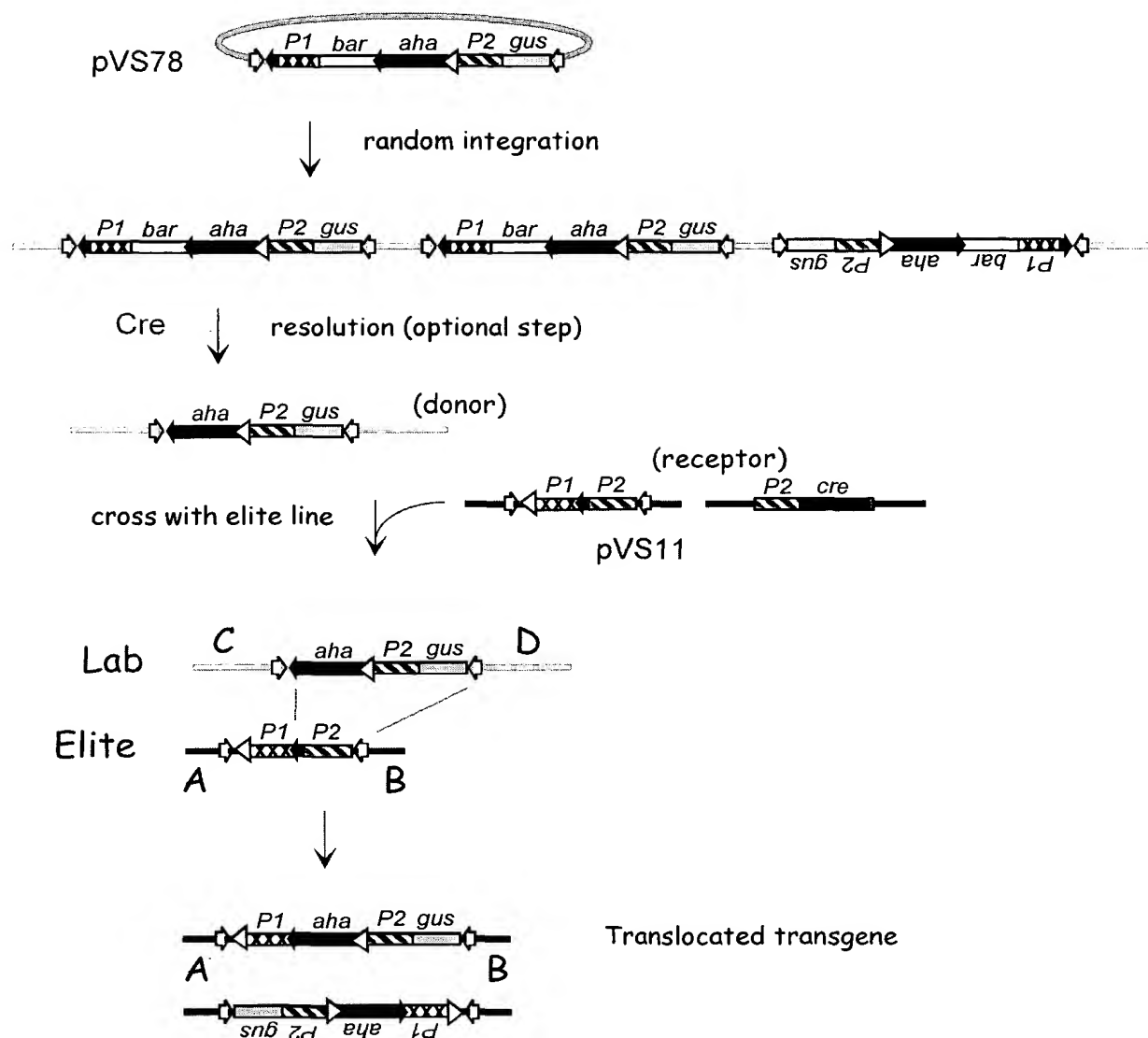## Transgene translocation using reversible recombination systems

P1 = Rice Actin promoter

P2 = Maize Ubiquitin promoter

◁ = FRT

◀ = loxP   ◁ = lox511